

A source of biological parts for Synthetic Biology. Xenologs?

Andrew Kuznetsov

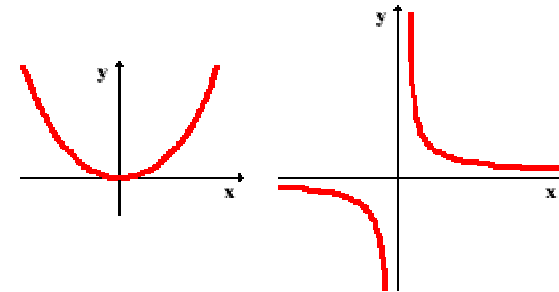
ATG:Biosynthetics,
Merzhausen, Germany

Content

- (1) Design principles in SB
 - Dissatisfaction with bio-bricks
- (2) Dialectical cycle: analysis <> synthesis
- (3) Environmental scanning. Sulfur metabolism in bacteria (an example)
 - reference microorganism
 - min set of genes for sulfur metabolism
 - mapping to environments
 - xenologs vs. orthologs
 - design and simulation

Design principles – MIT & Co

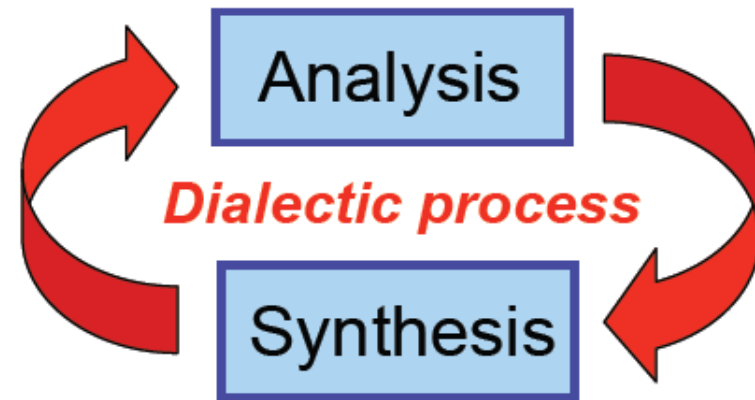
- An idea of SB is to build organisms that perform desired tasks, rather than to modify existing ones
- A major challenge for SB is to tackle complexity
 - top-down
 - bottom-up
- “Cell as an agent” may be a very suitable concept
 - design of agents with adaptive strategies
 - swarm algorithms
- Molecular logic and DNA embedded programming on the different levels
 - recombination
 - transcription
 - RNA interference
 - GTPases
 - Phosphorylation
- Separated genetic programs within different types of cells that work together can be used to avoid a cross talk
 - partition of genomes (incompatible plasmids)
 - compartments (membranes)
 - scaffold (DNA, RNA, proteins)
- Karman-Knight’s strategy:
information is turned off → abstraction → design



Dissatisfaction with the current bio-brick strategy

- new biological parts will lead to exponential growth of unspecific interactions in a target system – a cross talk
- standardization will lead to repeats in synthetic DNA and finally to recombinations – a genetic instability

Dialectical cycle in Freiburg



- “The central research idea of *bioSS* is to initiate and promote a dialectic process between scientists using analytical (dissecting) and synthetical (rebuilding) approaches in signalling research.”

Possible impact of bioinformatics on synthetic biology

- X-omics
- High-throughput sequencing technologies
 - 2d generation
 - Roche/454 FLX
 - Illumina/Solexa Genome Analyzer
 - Applied Biosystems SOLiD™ System
 - 3d generation
 - Helicos Heliscope™
 - Pacific Biosciences SMRT
- Understanding of biological complexity

CAMERA v1.3.2

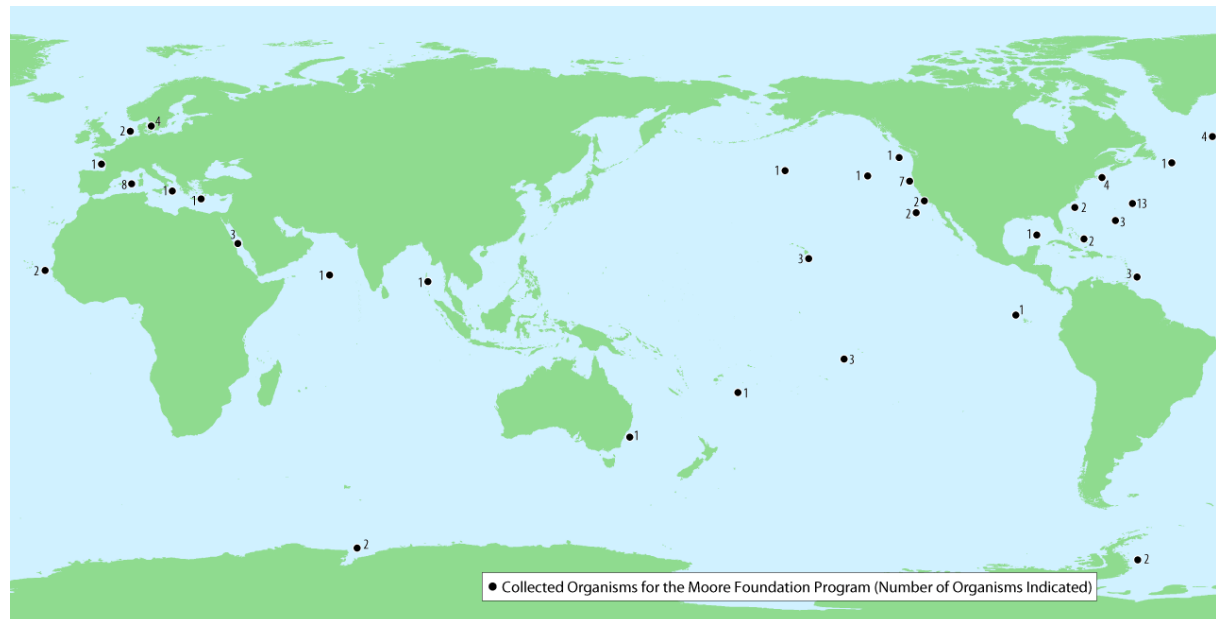


Community Cyberinfrastructure for Advanced
Microbial Ecology Research & Analysis

The Project was initiated
by the Gordon and Betty
Moore Foundation,
beginning in Jan 2006



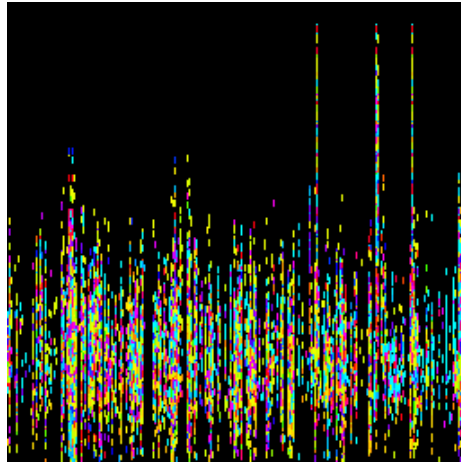
Sampling ('Sorcerer II', and other)



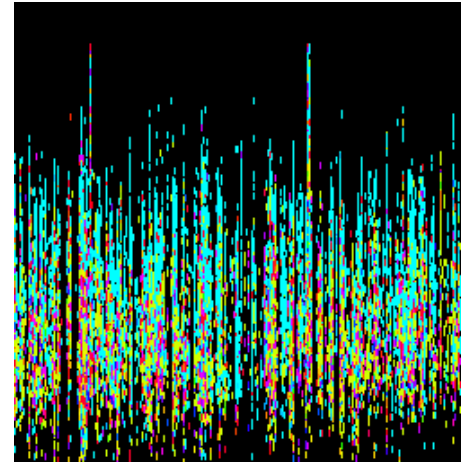
Databases that were used

- Collection from the Sorcerer II expedition
- Hawaii Ocean Time Series station which presents microbial genomes down to 4000 m
- Ionian abyssal plain, a deep flat basin between Sicily and Greece in the Eastern Mediterranean that is reached by H_2S
- Microbial community from deep-sea hydrothermal vent polychaete worm *Alvinella pompejana*
- Symbionts from another worm, Mediterranean gutless oligochaete *Olavius algarvensis*
- Microbes from Acid Mine Drainage biofilm at Iron Mountain, California
- Soil bacteria from Waseca County, Minnesota
- And other

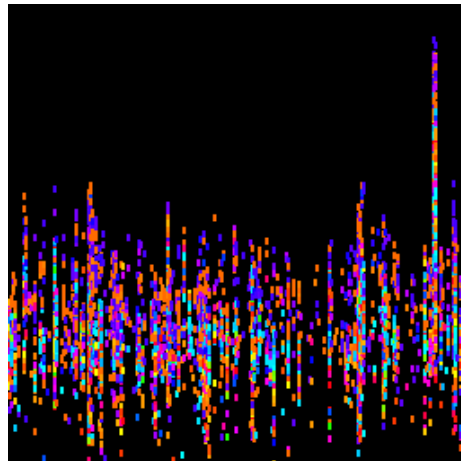
Fragment requirement plots of reference microorganisms



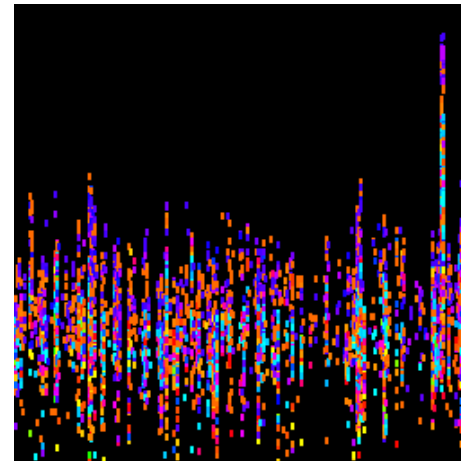
Thiomicrospira
crunogena
XCL-2
free-living bacterium
genome 2 427 674 nt
63 134 hits



Thiobacillus denitrificans
ATCC 25259
free-living bacterium
genome 2 909 749 nt
42 275 hits

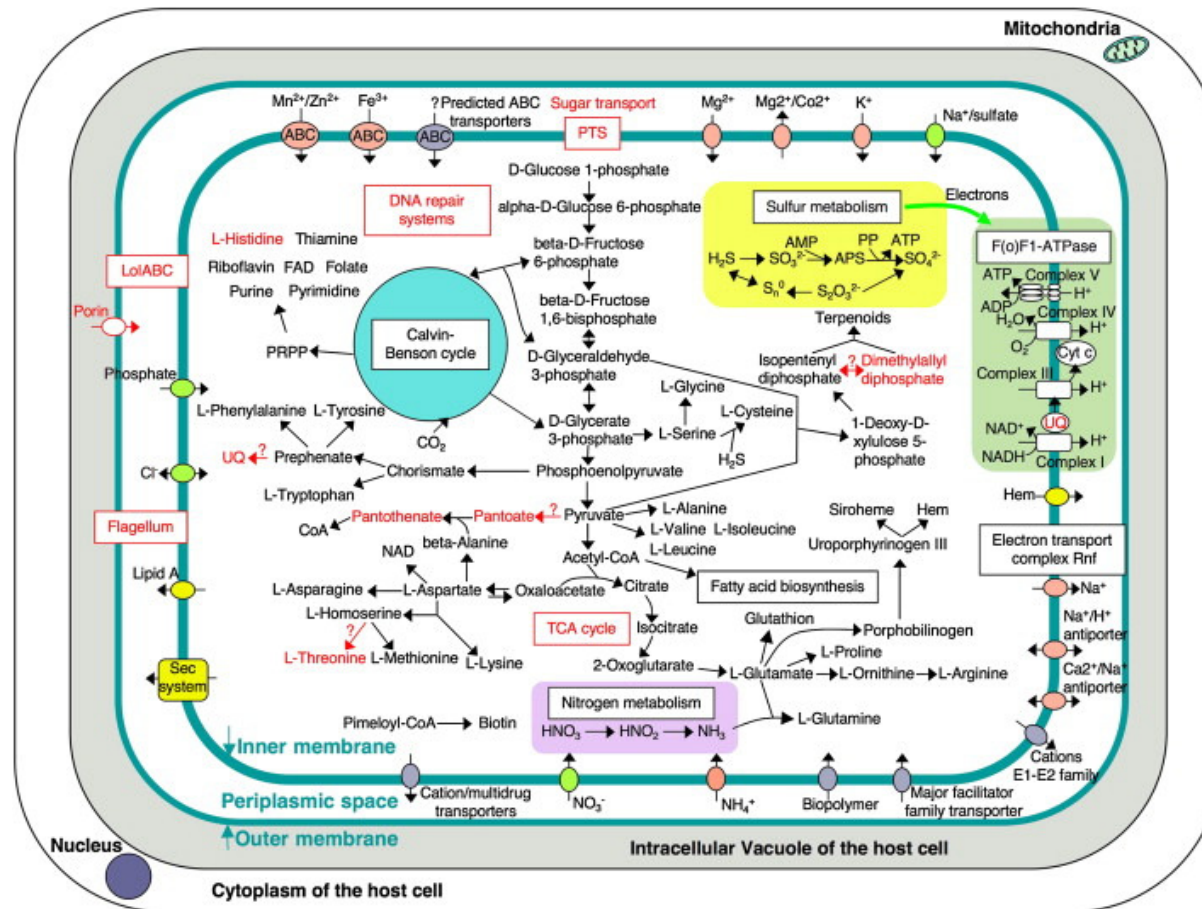


Vesicomysocius okutanii HA
host *Calyptogenia okutanii*
genome 1 022 154 nt
11 443 hits



Ruthia magnifica Cm
host *Calyptogenia magnifica*
genome 1 160 782 nt
11 160 hits

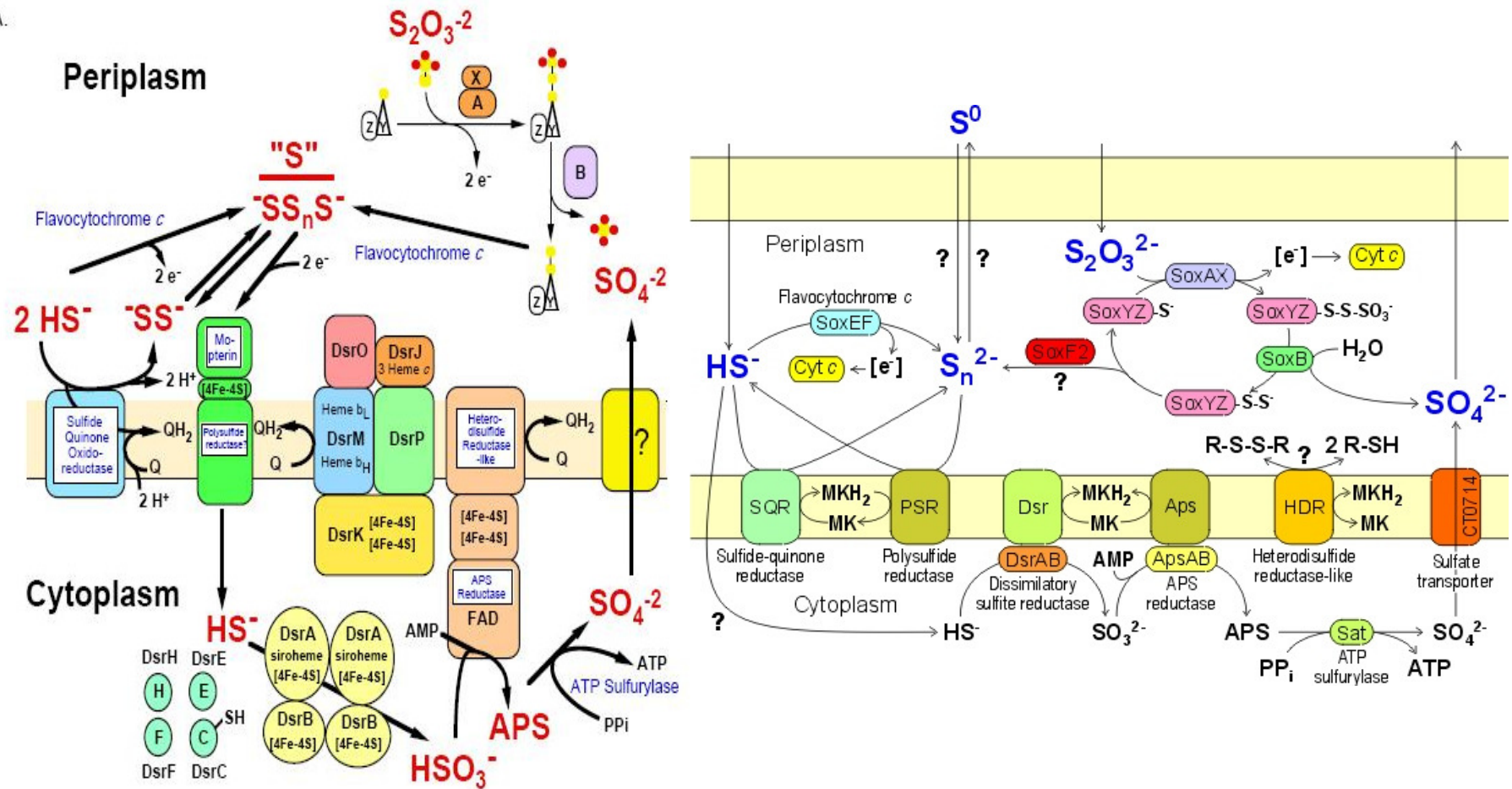
V. okutanii is a symbiont in a deep-sea clam, *Calyptogena okutanii* [Kuwahara et al, 2007]



Sulfur metabolism in bacteria

[lit. overview, KEGG, ERGO]

A.



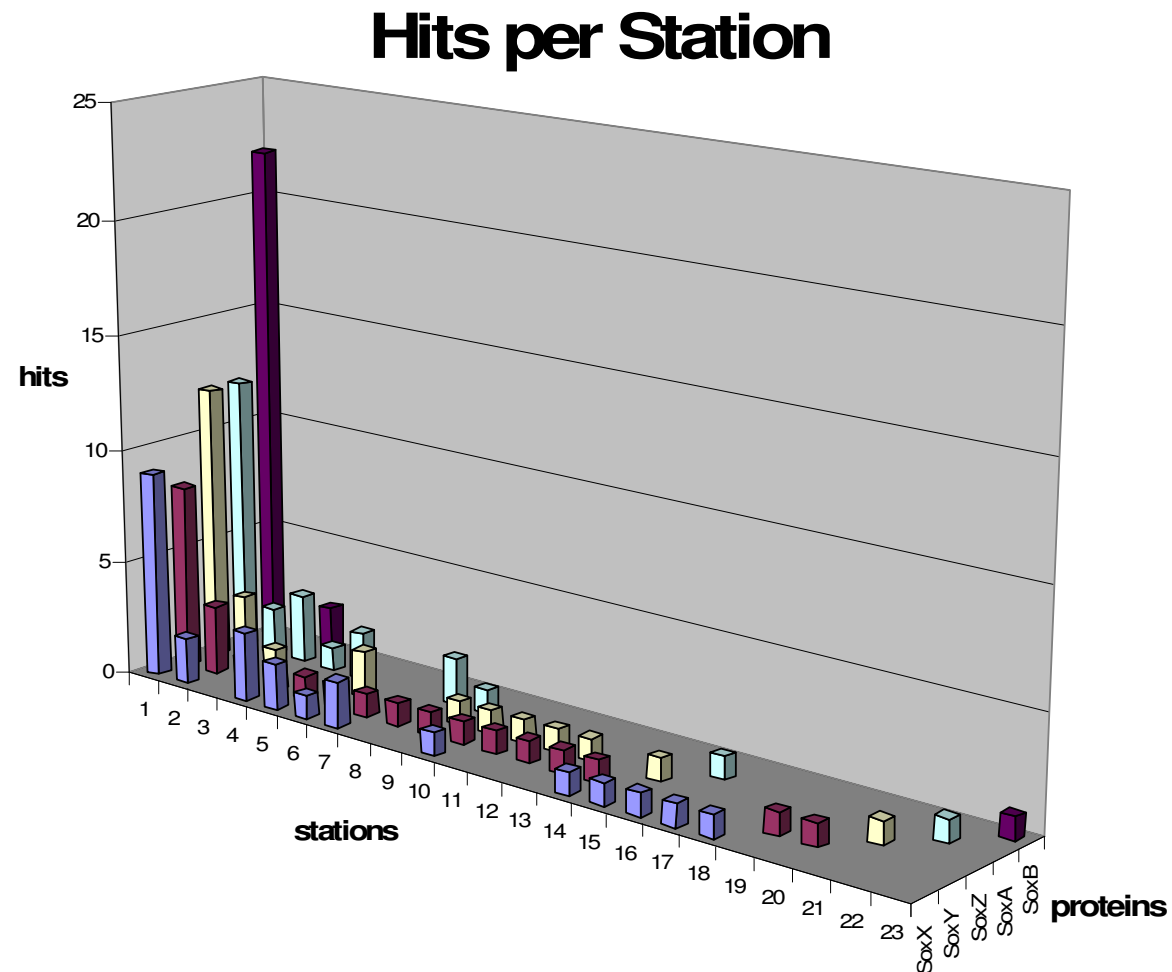
dissimilatory sulfite reductase (DsrAB), adenosine-5'-phosphosulfate reductase (APS), ATP sulfurylase (Sat) in cytoplasm, and Sox proteins in periplasm

The gene set from *V.okutanii* for a minimal sulfur metabolism

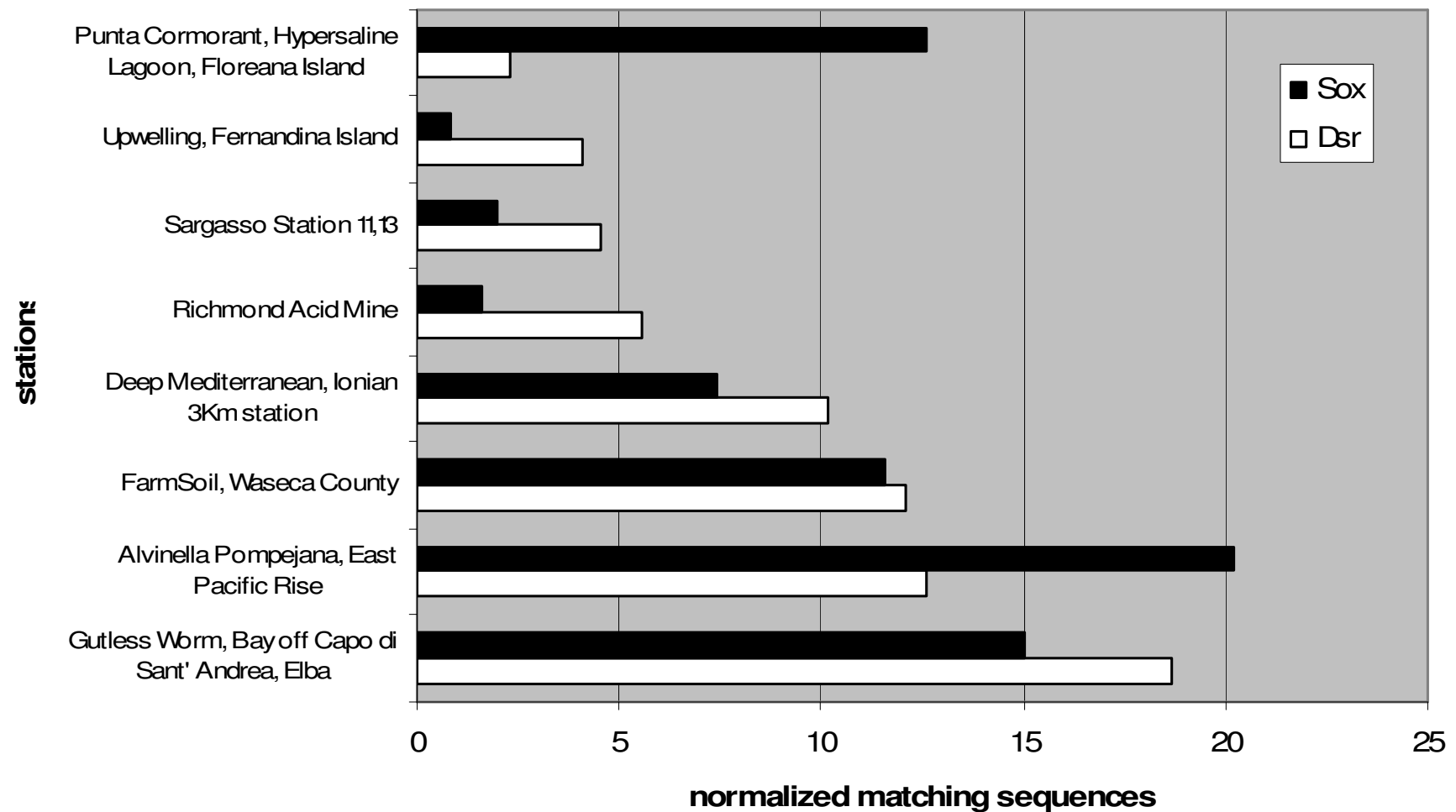
Product Name	Start	End	Strand	Length	Locus
ATP sulfurylase	98093	99301	+	402	<i>sat</i>
adenylylsulfate reductase membrane anchor	99516	100385	+	289	<i>aprM</i>
adenylylsulfate reductase β-subunit	100417	100896	+	159	<i>aprB</i>
adenylylsulfate reductase	100896	102779	+	627	<i>aprA</i>
sulfur oxidation protein SoxB	172596	174485	+	629	<i>soxB</i>
sulfur oxidation protein SoxA	770792	771607	-	271	<i>soxA</i>
sulfur oxidation protein SoxZ	771635	771937	-	100	<i>soxZ</i>
sulfur oxidation protein SoxY	771971	772414	-	147	<i>soxY</i>
sulfur oxidation protein SoxX	772425	772772	-	115	<i>soxX</i>
intracellular sulfur oxidation protein DsrR	817196	817537	-	113	<i>dsrR</i>
intracellular sulfur oxidation protein DsrP	818938	820140	-	400	<i>dsrP</i>
intracellular sulfur oxidation protein DsrO	820166	820897	-	243	<i>dsrO</i>
intracellular sulfur oxidation protein DsrJ	820894	821277	-	127	<i>dsrJ</i>
putative glutamate synthase (NADPH) small subunit	821307	823271	-	654	<i>dsrL</i>
intracellular sulfur oxidation protein DsrK	823327	824892	-	521	<i>dsrK</i>
intracellular sulfur oxidation protein DsrM	824894	825667	-	257	<i>dsrM</i>
intracellular sulfur oxidation protein DsrC	825744	826067	-	107	<i>dsrC</i>
intracellular sulfur oxidation protein DsrB	827224	828297	-	357	<i>dsrB</i>
intracellular sulfur oxidation protein DsrA	828373	829674	-	433	<i>dsrA</i>
rhodanese family protein	950273	950752	-	159	-
sulfide-quinone reductase	995954	997240	+	428	<i>sqr</i>

sox operon: SoxX, SoxY, SoxZ, SoxA, SoxB (CAMERA search)

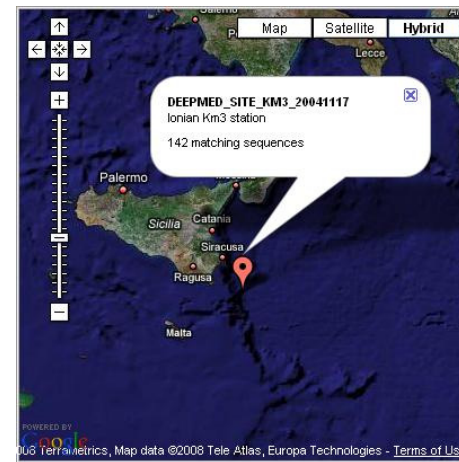
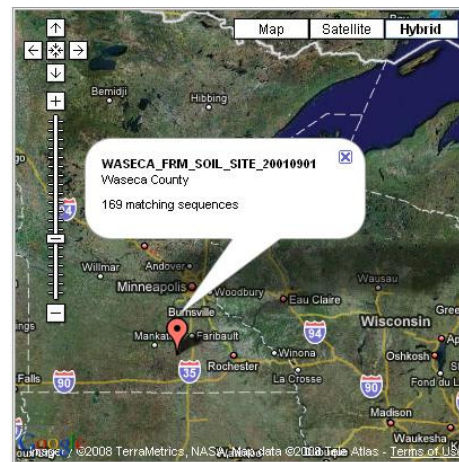
- 1 Punta Cormorant, Hypersalin...
- 2 Sargasso Station 11; Sargas...
- 3 Mangrove on Isabella Island
- 4 Yucatan Channel
- 5 Warm seep, Roca Redonda
- 6 Upwelling, Fernandina Island
- 7 Newport Harbor, RI
- 8 Gulf of Maine
- 9 Sargasso Stations 3
- 10 South of Charleston, SC
- 11 30 miles from Cocos Island
- 12 Hydrostation S
- 13 Cabo Marshall, Isabella Island
- 14 Off Key West, FL
- 15 Cape May, NJ
- 16 Wolf Island
- 17 Northern Gulf of Maine
- 18 134 miles NE of Galapagos
- 19 Outside Halifax, Nova Scotia
- 20 Rosario Bank
- 21 Sargasso Station 13
- 22 Gulf of Panama
- 23 Northeast of Colon



Stations with multiple Sox and Dsr hits, the normal values



location of stations with a plenty of Dsr matching sequences



Xenologs vs. Orthologs

- *dsr* reads generated trees incompatible with the corresponding 16S rRNA phylogeny
- due to the low quality of databases (random and partial DNA reads)?
- due to BLAST local alignment removes the most divergent regions from the sequences?
- or due to a lateral gene transfer? That is in agreement with [Klein et al, 2001; Boucher et al, 2003]

Bio-bricks for sulfur metabolism

sat-apr locus



sox locus



dsr locus



COSY_0913

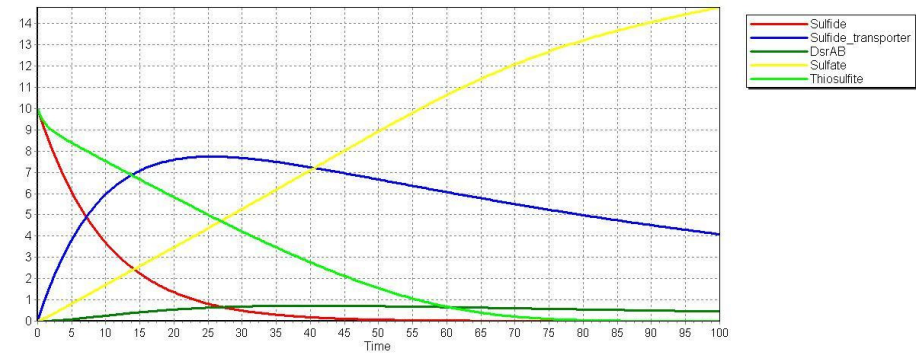
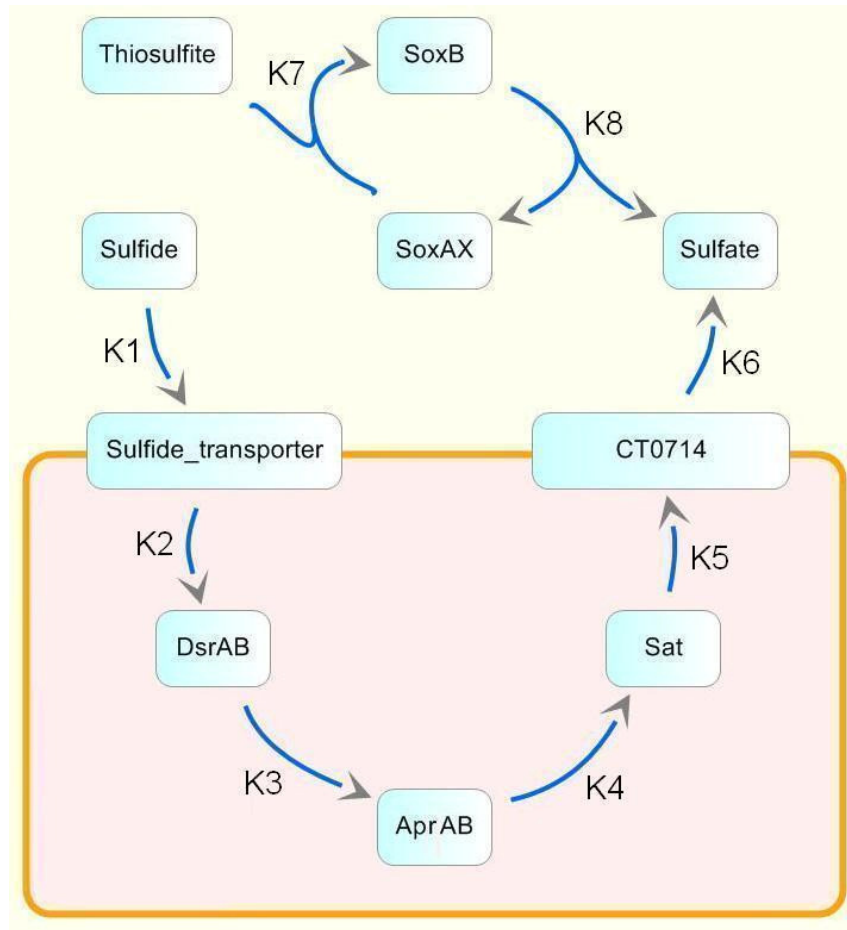


sqr gene (COSY_0953)

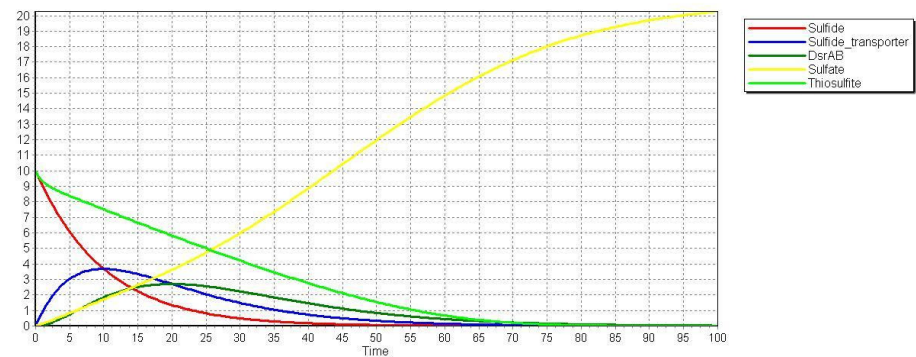


- Fragments of *V.okutunii* HA genetic map with genes coding sulfur metabolism

Simulation (1) within JDesigner

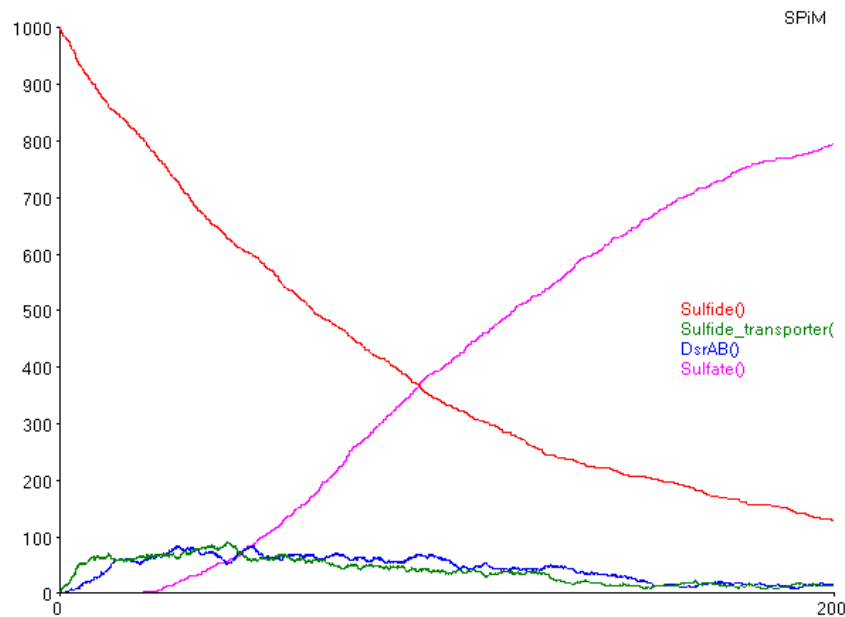
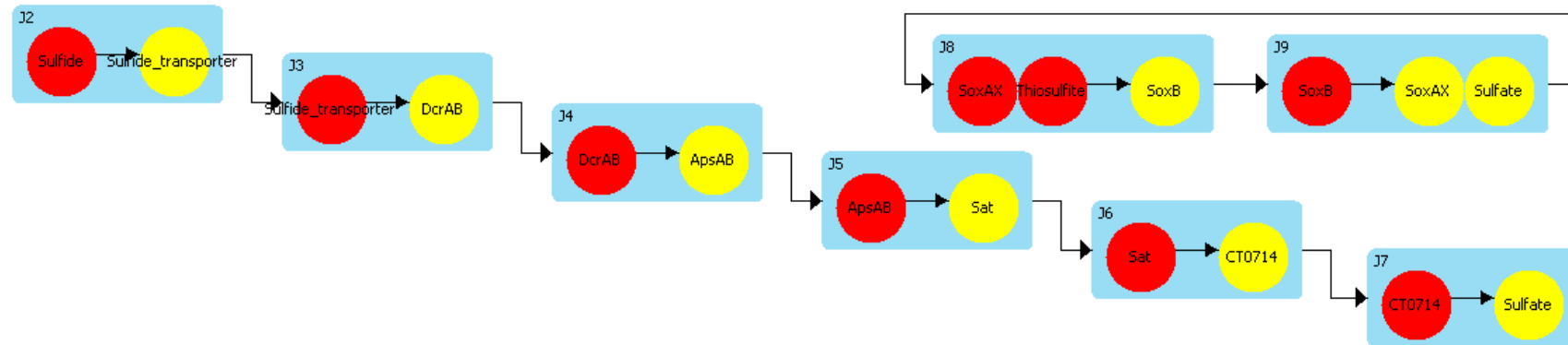


$k_2 = 0.01$

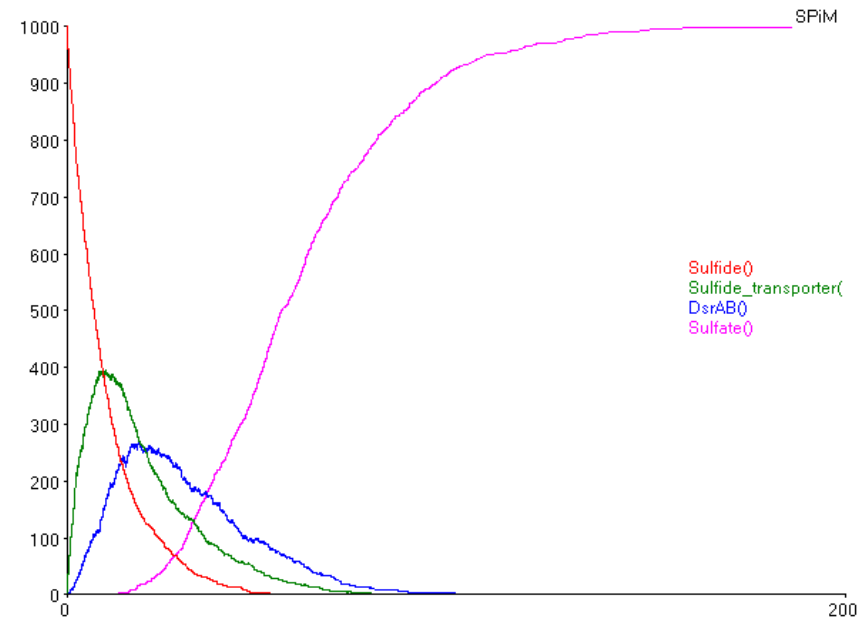


$k_2 = 0.1$

(2) within the stochastic pi-Machine



Sulfide delay 0.01



Sulfide delay 0.1

Conclusion

- *sat-apr*, *sox* and *dsr* locuses from *V.okutanii* are very suitable for design of sulfur metabolism
- xenologs as bio-bricks is an attractive idea which needs more detailed analysis

Thanks

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